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Review

Tissue engineering and cartilage

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Abbreviations: BMP, bone morphogenic protein; GDF, growth and differentiation factor; IGF, insulin like growth factor; TGF, transforming growth factor; PLLA, Poly (L-lactic) acid; PGA, polyglycolic acid; MSC, mesenchymal stem cell; RT-PCR, reverse transcriptase polymerase chain reaction; PEG, poly (ethylene glycol); ECM, extracellular matrix; PLGA, poly-lactic glycolic acid; MPa, mega pascals; Hz, hertz

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Human articular cartilage is an avascular structure, which, when injured, poses significant hurdles to repair strategies. Not only does the defect need to be repopulated with cells, but preferentially with hyaline-like cartilage.

Successful tissue engineering relies on four specific criteria: cells, growth factors, scaffolds, and the mechanical environment. The cell population utilized may originate from cartilage itself (chondrocytes) or from growth factors that direct the development of mesenchymal stem cells toward a chondrogenic phenotype. These stem cells may originate from various mesenchymal tissues including bone marrow, synovium, adipose tissue, skeletal muscle, and periosteum. Another unique population of multipotent cells arises from Wharton's jelly in human umbilical cords. A number of growth factors have been associated with chondrogenic differentiation of stem cells and the maintenance of the chondrogenic phenotype by chondrocytes in vitro, including TGFβ; BMP-2, 4 and 7; IGF-1; and GDF-5.

Scaffolds chosen for effective tissue engineering with respect to cartilage repair can be protein based (collagen, fibrin, and gelatin), carbohydrate based (hyaluronan, agarose, alginate, PLLA/PGA, and chitosan), or formed by hydrogels. Mechanical compression, fluid-induced shear stress, and hydrostatic pressure are aspects of mechanical loading found in within the human knee joint, both during gait and at rest. Utilizing these factors may assist in stimulating the development of more robust cells for implantation.

Effective tissue engineering has the potential to improve the quality of life of millions of patients and delay future medical costs related to joint arthroplasty and associated procedures.

Introduction

It has been well established that a full thickness defect of articular cartilage has significant limitations with respect to healing and repair. Cartilage is avascular, with injury typically followed by necrosis as opposed to the process of inflammation and repair found in vascularized tissues.[1] This fact requires innovative approaches and ideas to facilitate the regeneration of hyaline-like articular cartilage to avoid continued pain, joint arthroplasty, or arthrodesis.

"Tissue engineering is the regeneration and remodeling of tissue in vivo for the purpose of repairing, replacing, maintaining, or enhancing organ function, and the engineering and growing of functional tissue substitutes in vitro for implantation in vivo as a biological substitute for damaged or diseased tissues and organs."[2] Successful tissue engineering relies on multiple factors including obtaining appropriate cells for implantation; directing the development of those cells on a chondrogenic pathway using growth factors and/or cytokines; supporting the growing cells on a three-dimensional matrix (optimally biocompatible); and having that matrix remain in the cartilage defect, at least until healing is complete.[3]

Key concerns are prevalent with each of the aforementioned elements. First, it must be ensured that the implanted cells are immunoprivileged, or provide immunosuppressive agents to avoid rejection by the host immune system. Various growth factors, such as the bone morphogenic proteins (BMPs) are associated with both cartilage and bone development. It is crucial to halt the cascade of development at a cartilaginous stage, rather than having the implanted cells progress toward ossification and create islands of bone within the joint intraarticularly. Most polymer synthetic matrices have a tendency to degrade with a significantly acidic pH, proving harmful to the freshly implanted cells and to the other host tissues intraarticularly. Therefore a more biocompatible scaffold is optimal.[4]

There are many cell types that have been manipulated in vitro and subsequently implanted to repopulate a cartilage defect. These include chondrocytes, bone marrow derived mesenchymal stem cells; adipose, synovium, muscle, and periosteum derived stem cells; and cells derived from Wharton's jelly.[5] To properly manipulate these cells down the correct pathway "the right signals must be given at the right place and at the right time."[6] There are a multitude of growth factors that have been associated with cartilage regeneration including, but not limited to, BMP-2, 4, and 7, GDF-5, IGF-1 and TGFβ. These growth factors are introduced to the cell milieu in various manners, including viral vectors, non-viral vectors,

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nucleofection and direct delivery. The three-dimensional support structure is the final key to cartilage regeneration.

Various materials have been used to create scaffolds, some are protein based (collagen, fibrin and gelatin), others are carbohydrate based [hyaluronan, agarose, alginate, poly-L-lactic/polyglycolic acids (PLLA/PGA), and chitosan].[7] In addition to these classic scaffolds, newer forms of architecture including hydrogels are being used with more frequency. Finally, the mechanical environment these cell-containing scaffolds must reside in, in vivo, is much different than that of simple culture media in vitro. The human knee joint is exposed to multiple aspects of mechanical loading, including fluid-induced shear stress, mechanical compression, and hydrostatic pressure that may change the regenerative ability of the cells, the scaffolds, or the entire construct.[8-10]

The goal of this paper is to identify the keys to successful tissue engineering including the choice of cell type with a goal of simplified harvest from noncritical tissues, a suitable three-dimensional matrix, appropriate genetic enhancement, and proper attachment of cells and the matrix graft.

Cell Type

When faced with the challenge of a focal defect in articular cartilage the goal is to replace the damaged cartilage with hyaline or hyaline-like tissue. If accomplished, this regenerated cartilage will reduce the patient's symptoms, allow them to return to a productive level of function, and also allow for future treatment options, such as joint arthroplasty, should they become necessary, at the appropriate time.[11] In order to develop hyaline or hyaline-like tissue the foundation is based on the type of cells utilized and their ability to produce a chondrogenic phenotype.

Chondrocytes are an obvious choice for use as a population of cells to seed scaffolds for cartilage regeneration as these cells are already programmed to produce type II collagen and the associated extracellular matrix (ECM). Chondrocytes are typically harvested, full thickness, from non-load-bearing areas of healthy regions of articular cartilage. The cells are then cultured and expanded in vitro. Unfortunately, chondrocytes have the ability to lose their chondrogenic potential and dedifferentiate when going from a three-dimensional architecture to two-dimensional culture.[12] In order to produce hyaline-like cartilage this dedifferentiation and eventual type I collagen production must be avoided. Another concern is that with increasing age chondrocytes synthesize smaller and less uniform aggrecan molecules with less functional link proteins. These aging chondrocytes show declining synthetic and mitotic activities "with a decreased responsiveness to anabolic mechanical stimuli and growth factors."[13]

Mesenchymal stem cells (MSCs) can be obtained from a variety of sources, each of which has the potential to undergo chondrogenesis and facilitate cartilage regeneration. Recent studies have compared MSCs from various adult mesenchymal tissues, including synovium, periosteum, skeletal muscle, adipose tissue, and bone marrow.[5,14,15] The synovium, periosteum, and bone marrow MSCs all retained expandability after ten passages and showed improved chondrogenesis, compared to the adipose and skeletal muscle MSCs. We are already aware of the chondrogenic ability of synovium specifically with patients suffering from synovial chondromatosis.

Synovium can be obtained arthroscopically without causing donor site complications due to its high regenerative capacity. This eliminates one of the key issues associated with harvesting chondrocytes from healthy areas of articular cartilage. When comparing synovium to bone marrow MSCs, a cartilage pellet synthesized from synovium MSCs was significantly larger and heavier than that from bone marrow MSCs.[16] In addition with reverse transcriptase PCR (RT-PCR) it was noted that there was progressive expression of COL2A1 (a cartilage specific ECM molecule) and chondrogenic transcription factors Sox-9, 5 and 6.

As mentioned earlier, adipose tissue has the ability to yield mesenchymal stem cells that may be directed toward a chondrogenic phenotype. Benefits of this tissue include easy accessibility, minimal morbidity upon harvest, and a clinically relevant number of stem cells can be obtained, limiting the need for expansion.[17] Another study has shown that the frequency of colony forming unit fibroblasts was three times greater in adipose tissue than in bone marrow.[18] There has been an attempt by Helder and colleagues to create a single surgical procedure where the stem cells can be harvested, directed to a certain lineage, chondrogenic via BMP-7, or osteogenic via BMP-2, then seeded on a scaffold and implanted. This group found that stimulation with the growth factors for fifteen minutes yielded the same results as stimulation for four days.[17] This individual study may direct future research towards creating a one-step surgical procedure that includes harvesting cells, directing those cells toward a chondrogenic phenotype, seeding the cells on a scaffold, and then implanting that scaffold arthroscopically.

The challenge of cartilage being an avascular structure with localized areas of hypoxia and ischemia adds an aspect of complexity with respect to transplanted cells. The mesenchymal stem cells arise from an area that is highly vascular and are generously supplied with nutrients. The transplanted cells must now learn to survive and function in an ischemic host environment so they may contribute to chondral repair. Ischemia induces apoptosis in the MSCs, but only after 36–48 hours and the MSCs typically begin their differentiation prior to this event.[19]

Human umbilical cord perivascular (HUCPV) cells are derived from the primitive connective tissue of Wharton's jelly.[20] They are a mesenchymal precursor cell population that has a high frequency of colony forming unit fibroblast (CFU-F) deriving cells. These cells express neither class I nor class II major histocompatibility (MHC) antigens highlighting their immunoprivileged status. While umbilical cord blood can yield MSC-like cells (1:200 million cells), the CFU-F frequency in the HUCPV cells is 1:300 cells obtained. These cells also were noted to spontaneously form bone nodules in non-osteogenic culture conditions. A goal therefore would be to derive a chondrogenic lineage facilitated by cartilage specific growth factors.

Two additional studies have confirmed the utility of the HUCPV cells. Baksh et al., illustrated that these cells did not experience contact-inhibited cell growth at twenty days, showed better osteogenic and adipogenic potential, but similar chondrogenic potential compared to bone marrow MSCs when exposed to similar growth factors.[21] Troyer and Weiss compared these cells to chondrocytes and found that the Wharton's jelly derived cells had improved outcomes with respect to collagen type II and glycosaminoglycan production after four weeks of culture.[22]

It is clear that there are a variety of cell sources available to facilitate cartilage regeneration. The goal is to find a population that is easily accessible, does little harm to healthy tissues, is easily expandable, and retains the chondrogenic phenotype in culture prior to transplantation.

Growth Factors

With cells being the starting point for cartilage regeneration there must be a way to direct their differentiation down a pathway that is primarily chondrogenic to attain the goal of hyaline cartilage formation. In a review,[23] our group has shown that Transforming Growth Factor β (TGFβ) facilitates cartilage regeneration that was well integrated into adjacent tissue; Bone Morphogenic Proteins (BMPs) can stimulate mitosis and matrix production in chondrocytes, and trigger MSCs to differentiate and maintain a chondrogenic phenotype. Insulin Like Growth Factor (IGF-1) stimulates mitosis, cell differentiation and matrix synthesis in articular chondrocytes, while inhibiting the basal rate of matrix degradation in normal articular cartilage. Another growth factor that is vital in the chondrogenic lineage is Growth and Differentiation Factor 5 (GDF-5), which plays a role in chondrocyte, fibroblast and mesenchymal cell expansion.[24]

When expanding chondrocytes in culture, TGFβ will increase the cartilage expression of type II collagen and will produce a significantly greater amount of DNA and glycosaminoglycans. BMPs 2 and 4 will stimulate cartilage formation and GDF-5 will yield an enhanced number of prechondrogenic precursors and an elevated level of Sox-9.[12,24] BMP-4 is specifically instructive to chondrogenesis by inducing mesenchymal cells to become chondroprogenitors and promotes their differentiation into mature chondrocytes.[25]

In a study examining the effects of GDF-5 and BMP-4 on embryonic limb bud mesenchymal cells, it was found that GDF-5 promoted mesenchymal cell condensation and cartilage nodule formation more significantly than BMP-4.[25] BMP-4 increased Sox-9 levels at 24-48 hours, but they then returned to control levels, while the GDF-5 related increase of Sox-9 remained elevated over controls. The authors concluded that BMP-4 is related to an early phase of cell fate determination, while GDF-5 is focused on a later phase of differentiation.

Insulin like growth factor 1 (IGF-1) is another potent growth factor that has been shown to have anabolic effects on matrix synthesis when added to chondrocytes. Specifically, it has been shown to increase the synthesis of major cartilage proteins (proteoglycans and type II collagen) while inhibiting the degradation and release of proteoglycans. Fortier et al., cultured mature chondrocytes in the presence of IGF-1 and found increased levels of type II collagen and aggrecan mRNA compared to controls, with the chondrocytes maintaining a round phenotype without cluster formation. There was also a dose dependent increase in total glycosaminoglycan (GAG) and total collagen and a lack of type I or IIA procollagen, indicating no dedifferentiation.[26]

Additional studies by the same group illustrated that defects exposed to IGF-1 were better attached to subchondral bone, and had an improved chondrocyte population with a significantly improved histological score.[27] Chondrocytes transduced with IGF-1 were found to have a 100 fold increase in collagen type II at four weeks, with an improved filling of defects, more hyaline-like tissue covering the lesion at eight weeks and improved histological scores at four and nine weeks compared to controls.[28]

Scaffolds

The ultimate goal in tissue engineering is to recreate the native architecture and function of the targeted tissue. A material must be able to support the growth and expansion of either chondrocytes or mesenchymal stem cells, facilitate their free diffusion and movement throughout the structure while remaining stiff enough to mimic native articular cartilage.

Hydrogels are a three-dimensional network of hydrophilic polymers that absorb a large quantity of water as well as biological fluids.[29] In addition, poly (ethylene glycol) (PEG) based hydrogels are used for cartilage tissue engineering scaffolds because of their water content, elasticity, biocompatibility and ability to permit diffusion of nutrients and bioactive molecules.[30-32] Sharma et al., found a combination of mesenchymal stem cells loaded on hydrogels containing both hyaluronic acid and TGF-β3 yielded more proteoglycan, and type II collagen, less type I and X collagen, and improved expression of cartilage specific genes (aggrecan) when compared to hyaluronic acid or TGFβ3 alone.[33]

Another important requirement of an optimal scaffold is the ability to remain stable until a continuous network of ECM is present and then degrade as the network matures. These scaffolds must be biocompatible and not degrade into harmful residues that will diminish the replicative capacity of the seeded cells. A third attribute is the compressive stiffness of the scaffold should resemble that of native articular cartilage so as not to wear differently than the surrounding tissue.[34] In a study examining the difference in chondrogenic potential of bone marrow and adipose stem cells on two different hydrogels, agarose and self-assembling peptide, it was found that bone marrow MSCs had an increased production of ECM in the presence of TGFβ and the peptide hydrogel was a better host than was agarose.[35]

Poly-lactic glycolic acid (PLGA) is a synthetic material that has excellent biocompatibility and biodegradability. Chitosan is a substance extracted from natural sources and also has excellent biocompatibility and is used in wound healing.[36] Chitosan has smaller pores and a higher density than PLGA. Porosity is a key factor in a scaffold as cells need to migrate freely throughout and become supplied by serum materials. The scaffolds were seeded with chondrocytes and implanted subcutaneously in nude mice. At 8 and 12 weeks the chitosan maintained volume while the PLGA scaffold was almost completely absorbed. At four weeks the PLGA scaffold had good cartilage development, but it was absorbed by 16 weeks. At 16 weeks there was degradation of the chitosan scaffold with the development of mature cartilage.[36] The porosity of the chitosan may have been a factor in the delayed development of cartilage, however its durability allowed a mature network of ECM to be laid down.

Hyaluronic acid is a naturally occurring molecule present in the ECM of articular cartilage.[37] Comparing hyaluronic acid sponges (HYAFF-11®) to ACP sponges (crosslinked hyaluronan), and ceramics, the HYAFF-11® (benzylated hyaluronan) sponges have a much slower turnover rate, which may explain why the ACP sponges degraded by 3–6 weeks and had no bone or cartilage in surgical pockets and the HYAFF-11® sponges had bone, cartilage, and fibrous tissue. Also, at three weeks the amount of tissue in the HYAFF-11® sponges was double that found in ceramics. The HYAFF-11® sponges are highly porous, which ensures cell to cell contact and enhanced chondrogenic differentiation.[38] In addition, these scaffolds have excellent cell retention and rigidity, which ensures easy manipulation during implantation.

Mechanical Environment

Maintaining a chondrogenic phenotype in vitro is the first step towards obtaining an acceptable combination of cells and scaffold for cartilage regeneration. However, to be able to apply these constructs in vivo, one must confirm that they will remain unified and effective when exposed to normal articular conditions. Subjecting the constructs to biomechanical stresses prior to implantation may also facilitate the appropriate phenotype.

During normal gait the articular surface is subjected to many forces including, but not limited to hydrostatic pressure. Chondrocytes are therefore adapted to live under pressurized conditions.[10] When chondrocytes were seeded on agarose molds and subjected to hydrostatic pressures of 10 MPa, at 1 Hz, applied for four hours per day, five days per week for eight weeks, there was a significant increase in total collagen and the chondrocytes remained spherical and in lacunae. Over the eight weeks the total amount of GAG decreased in the controls to a greater degree than the pressurized chondrocytes.[10] A similar study revealed that when chondrocytes were cultured in three-dimensional agarose gels and subjected to a hydrostatic pressure of 5 MPa for four hours there was a significant increase in GAG synthesis and a four-fold increase in aggrecan mRNA synthesis.[39]

With porosity being a critical aspect of scaffold architecture, assisting the flow of nutrients and growth factors through the scaffold should improve chondrogenesis. To that end Pazzano et al., compared chondrogenesis of chondrocytes seeded on PLLA/ PGA scaffolds in static and perfused bioreactors.[40] The investigators showed the perfused bioreactor yielded a 118% increase in DNA content, a 184% increase in GAG concentration at four weeks, and a 130% increase in hydroxyproline content compared to static controls (all results significant).

Fluid-induced shear stress has also been shown to improve chondrocyte proliferation on cells grown in monolayer.[8] When chondrocytes were isolated and plated in monolayer on slides and exposed to shear stress (3.5 Pa for 96 hours), they overgrew the monolayer, while those in static culture remained in monolayer. Those exposed to the shear stress also secreted a significantly greater amount of TGFβ and upon histological examination, the cells that formed on top of the monolayer were more rounded, or of a chondrogenic phenotype.

Chondrocytes are intended to grow and develop intraarticularly; it therefore is intuitive that recreating their mechanical environment will provide them with cues to secrete appropriate growth factors and differentiate along the proper pathway.

Conclusion

It is clear that tissue engineering is a multidisciplinary field with many factors leading to the desired outcome. With respect to cartilage regeneration, a foundation of cells, either chondrocytes or mesenchymal stem cells must be readily available for harvest causing as little damage as possible to healthy tissues. Various growth factors that trigger development of a chondrogenic phenotype are necessary, while preventing completion of the cascade toward bone formation. Scaffolds with an appropriate architecture for support while maintaining porosity for diffusion are necessary to provide the cells a stable location to grow. Finally the mechanical environment that the cells will eventually be implanted should not be forgotten as a key to chondrogenic differentiation.

Tissue engineering has the potential to save millions of dollars in future healthcare costs, especially with the aging of the population. It is a field that, with respect to Orthopedic Surgery, can impact a patient's quality of life like no other field in medicine.

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